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# *Indian Standard*

# METHOD FOR DETERMINATION OF AVAILABLE LYSINE IN FOODS

( First Reprint MAY-1984 )

UDC 664 : 543 : 547.466.46

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**INDIAN STANDARDS INSTITUTION**
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

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*November* 1976

# *Indian Standard*

# METHOD FOR DETERMINATION OF AVAILABLE LYSINE IN FOODS

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# *Indian Standard*

# METHOD FOR DETERMINATION OF AVAILABLE LYSINE IN FOODS

## 0. FOREWORD

**0.1** This Indian Standard was adopted by the Indian Standards Institution on 31 August 1976, after the draft finalized by the Food Hygiene, Sampling and Analysis Sectional Committee had been approved by the Agricultural and Food Products Division Council.

**0.2** Available lysine content is considered as the most important chemical ( non-biological ) indicator of nutritional quality of protein because low values reflect loss due to heat processing. Since lysine is a limiting amino acid, its fortification should be viewed in the light of the concept of suitable and reliable method of analysis which gives repeatable and reproducible results. There are mainly two methods, namely, chemical and microbiological, for analysis of lysine. Of these, chemical method is more precise and accurate. This standard, therefore, prescribes chemical method for analysis of available lysine. It is expected that this method will help in achieving uniformity in the analysis of lysine thereby facilitating uniform interpretation and comparison of results.

**0.3** In the preparation of this standard, considerable assistance has been derived from a number of books and publications. However, the method included in this standard is predominantly that which has been tried in various laboratories in the country and is mainly based on practical experience gained within the country.

**0.4** In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

## 1. SCOPE

**1.1** This standard prescribes chemical method for determination of available lysine in foods.

## 2. TERMINOLOGY

**2.0** For the purpose of this standard, the following definition shall apply.

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*Rules for rounding off numerical values ( *revised* ).

**2.1 Available Lysine** — That fraction of lysine which reacts with fluorodinitrobenzene ( FDNB ) associated with biologically active fraction of lysine.

## 3. PRINCIPLE

**3.1** The method is based on the conversion of lysine residues with the reactive epsilon amino groups in food proteins into yellow epsilon dinitrophenyl ( DNP ) lysine by treatment of the material with FDNB and colorimetric estimation of the DNP lysine obtained by a subsequent acid hydrolysis.

## 4. APPARATUS

**4.1 Autopipette or Suitably Graduated Pipettes**

**4.2 Burette** — 25 ml capacity, graduated to 0·05 ml.

**4.3 Conical Flasks**

NOTE — It is not advisable to clean the flasks with chromic acid; but, if this is done, they should be soaked in dilute sodium hydroxide and rinsed with distilled water thoroughly.

**4.4 Photoelectric Colorimeter** — absorbance at 435 nm.

**4.5 Round-Bottom Flasks** — 100 or 150 ml capacity with 8 cm long necks and standard joint 24-29 for fitting to condensers.

**4.6 Stoppered Test Tubes** — graduated at 10 ml.

**4.7 Water-Bath** — for temperature of 100°C.

## 5. REAGENTS

**5.0** Unless specified otherwise, pure chemicals and distilled water ( *see* IS : 1070-1960* ) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the test results.

**5.1 Mono F-N-Dinitrophenyl Lysine Hydrochloride Monohydrate ( DNPL )** — Dissolve 314 mg of lysine in 250 ml of 8·1 N hydrochloric acid. Dilute 10 ml standard DNPL solution with water to 100 ml. Use this diluted solution as a standard for the routine tests. A 2-ml aliquot contains the equivalent of 0·1 mg of lysine when diluted to 10 ml has a net absorbance of about 0·4 at 435 nm in a 1-cm cuvette.

NOTE — This compound dissolves slowly. It is, therefore, desirable to allow a day for the process.

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*Specification for water, distilled quality ( *revised* ).

**5.2 1-Flouro-2,4-Dinitrobenzene ( FDNB )** — If the FDNB is solid, place the bottle in warm water for a few minutes before dispensing it with an autopipette. Prepare FDNB solution in ethanol every day. Each sample will require 12 ml of ethanol solution containing 0·3 ml of FDNB. Actual measurement of FDNB may not be essential.

NOTE — It is a vesicant and, therefore, disposable gloves of polyethylene, not rubber, should be worn during its use. All operations involving the use of the vesicant including its addition to the sample may be performed on a sheet of paper which may be disposed of later.

**5.3 Methoxycarbonyl Chloride ( MCC )** — 8 percent ( *m*/*v* ).

**5.4 Sodium Bicarbonate Solution** — 80 g in one litre of distilled water.

**5.5 Buffer Solution** — 19 parts of 8 percent sodium bicarbonate and 1 part of 8 percent sodium carbonate adjusted suitably to *p*H 8·5.

**5.6 Hydrochloric Acid** — concentrated, 1 N and 8·1 N.

**5.7 Diethyl Ether** — peroxide free.

**5.8 Phenolphthalein Indicator Solution** — 0·1 g in 100 ml of 50 to 60 percent ethanol.

## 6. PROCEDURE

**6.0** Carry out the procedure in duplicate and away from direct or strongly reflected sunlight.

**6.1** Take 50 g of the material finally ground to pass through 425-micron IS Sieve ( IS : 460-1962* ). Take samples for the determination of nitrogen also in duplicate at this time. Then take two portions, each containing an estimated 30 to 50 mg of nitrogen into round-bottom flasks and to each add 8 ml of 8 percent sodium bicarbonate. Shake gently to disperse the material and leave for 10 minutes. The sample should not be widely scattered. Add 0·3 ml FDNB, previously dissolved in 12 ml of ethanol to each flask, stopper the flask and shake gently on a mechanical shaker for 2 hours. Remove the stoppers and allow the flasks to stand in boiling water to remove the ethanol. Add immediately 24 ml of 8·1 N hydrochloric acid to one flask. To the other flask add only 12 ml of 8·1 N hydrochloric acid. Then add exactly 12 ml of a solution of DNPL in 8·1 N hydrochloric acid, a quantity that contains the equivalent of 8 mg lysine which serves as an internal standard. Add DNPL after the FDNB reaction has been stopped by the acid; otherwise it will be converted to bis-DNPL which will be eliminated by extraction with ether. Reflux the contents of the flask gently for 16 hours. Disconnect the flasks after the condensers have been washed with water. Cool the flasks in ice for 2 hours to keep for easy filtration. Filter the contents through a Whatman filter paper No. 41 or equivalent, with water washings into a 250-ml volumetric flask. Make the filtrate

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*Specification for test sieves ( *revised* ).

to the volume and mix. Dilute an aliquot of the filtrate so that 2 ml of the dilute material contains an estimate of approximately 50 $\mu$g of the available lysine.

NOTE — A precipitate of dinitrophenol may form. The precipitate should be allowed to settle and avoided during the pipetting. It should not be transferred during the next stage, but should be removed by ether.

**6.2** Pipette 2 ml from each diluted filtrate into each of the two glass-stoppered test tubes marked 'A' and 'B', and a small conical flask marked 'C'. Extract the contents of the tubes thrice with 5·0 ml of ether. Discard as much of the ether as safely as could be by taking it off with a dropping pipette or autopipette. Hold the tubes in hot water (about 80°C) until effervescence from the residual ether had ceased and cooled off. Make up tube 'A' to 10 ml with 1 N hydrochloric acid and keep for the final readings.

**6.3** Dilute the contents of flask 'C' with water and titrate with 2 N sodium hydroxide, using a drop of phenolphthalein indicator. Note the quantity of sodium hydroxide needed and then add the same volume to tube 'B', followed by 2 ml of buffer solution. Further additions should be continued without pause as DNP-compounds are less stable at this *p*H. Add methoxy carbonyl chloride (0·045 to 0·055 ml permissible) and shake the tube vigorously to disperse and dissolve the compound. After 5 to 10 minutes, add carefully 0·75 ml of concentrated hydrochloric acid. Extract again the contents 4 times with 5 ml ether. Discard the ether washings. Evaporate the residual ether in the aqueous layer by standing the tube in hot water bath. Cool the tube and make up the contents to 10 ml with water.

**6.4** Read the absorbances of the contents of tubes 'A' and 'B' at 435 nm against water. Reading 'A' minus reading 'B' (the blank) is the net absorbance attributable to DNP-lysine.

## 7. CALCULATIONS

### 7.1 Available Lysine in g per 100 g of Material

$$= \frac{(A - C)}{(B - D) - (A - C)} \times \frac{X}{W} \times 100 \times \frac{40}{100}$$

where

$A$ = sample reading,
$B$ = sample and extra DNP-lysine,
$C$ = blank for sample,
$D$ = blank for sample and extra DNP-lysine,
$W$ = sample mass equivalent in g of 2-ml aliquot,
$A - C$ = correct sample reading, and
$B - D$ = reading for extra DNP-lysine ($X$ gram) in 2-ml aliquot.

NOTE — The method is not suitable for measuring added lysine or free lysine in a hydrolysed product. This yields di-DNP-lysine which is lost in the first ether wash.